J. Pharm. Pharmac., 1973, **25**, 876–880 *Received May* 16, 1973

The effect of carbenicillin on the haemostatic mechanism

D. A. LEDERER, T. DAVIES, G. CONNELL, J. A. DAVIES AND G. P. MCNICOL

University Department of Medicine, The General Infirmary, Leeds, Yorkshire, U.K.

Carbenicillin has no effect on the thrombin time, partial thromboplastin time, prothrombin time, platelet factor 3 availability, fibrinogen and plasminogen levels, Factor XIII, fibrin plate lysis or euglobulin lysis time *in vitro* in concentrations from 20–1280 μg ml^{-1}. After incubation with plasma, carbenicillin inhibited platelet aggregation to ADP at all the concentrations examined (20–1280 μg ml^{-1}). The same coagulation tests were unaffected 30 min and 4 h after intravenous administration of 5 g of carbenicillin to six normal subjects, though some impairment of platelet response to ADP occurred in three subjects. This impairment of platelet function is considered unlikely to contribute towards a bleeding tendency in normal subjects but might perhaps be a contributory factor in haemostatic failures in patients with uraemia.

Carbenicillin (disodium carboxybenzyl/penicillin) is a semisynthetic penicillin of particular use in treating infections due to species of *Proteus* or *Pseudomonas*, for which high concentrations are required. Therefore, large doses of carbenicillin providing blood levels of up to 200 μg ml^{-1} are recommended (Bodey & Terrell, 1968; Brumfitt, Percival & Leigh, 1967; Acred, Brown & others, 1967; Marks & Eickhoff, 1970).

A tendency to bleed developing in patients receiving carbenicillin was first reported by Lurie, Ogilvie & others (1970a) and subsequently by McClure, Casserly & others (1970), Waisbren, Evani & Ziebert (1971) and Yudis, Mahood & Maxwell (1972). Lurie, Gold & others (1970b) also reported *in vitro* effects of carbenicillin on coagulation using concentrations of 6000 μg ml^{-1} and concluded that the drug affected fibrinogen-fibrin conversion. McClure & others (1970) considered the tendency to bleed to be due to an effect of carbenicillin on platelet function as evidenced by depressed platelet aggregation in response to ADP. He also showed similar effects with platelet rich plasma incubated for 2 h *in vitro* with 700 μg ml^{-1} carbenicillin.

Most reports of abnormal bleeding during carbenicillin treatment have concerned patients who were either receiving other drugs, including a variety of antibiotics, or who had impaired renal function. It was therefore decided to investigate the effect of carbenicillin on the haemostatic mechanism *in vitro* and in normal volunteers.

MATERIALS AND METHODS

In vitro

Pooled human plasma was obtained from normal volunteers. Carbenicillin was dissolved in distilled water and added to pooled plasma to achieve final concentrations of 20, 40, 80, 160, 320, 640 and 1280 μg ml^{-1}. Coagulation studies were made immediately after the addition of carbenicillin and repeated after incubation at 37° for 2 h. Control experiments were made on the pooled plasma at both times.

In vivo

Six normal volunteers, none of whom was receiving any drug, had fasting blood samples and bleeding times measured. Carbenicillin, 5 g dissolved in 15 ml of sterile distilled water, was administered by slow intravenous injection over 5 min into an antecubital vein. Further blood samples were taken 30 min and 4 h after the administration of the drug. The bleeding time was measured after 4 h. Haemostatic function was assessed by the methods described below.

METHODS

Factor XIII was assessed by clot stability in urea and acetic acid as described by Lorand (1950).

Platelet aggregation was investigated by the turbidometric method described by Born (1962). Platelet aggregation was measured in response to ADP (2 μM final concentration), to adrenaline (5 μM final concentration) and collagen. Change in optical density was measured 30, 60, 90, 120 and 150 s after addition of the aggregating agent. The initial rate of aggregation was determined as the slope of the steepest portion of the optical density curve.

Carbenicillin levels were measured by a microbiological method using *Pseudomonas aeruginosa* (Ellsworth, B.R.L. 1973 NCT C 10 490) as assay organism.

Statistical analysis. Values obtained before and after the administration of carbenicillin were assessed for statistical significance using the paired *t*-test.

The other methods used in the coagulation assays are described in the footnote to Table 1.

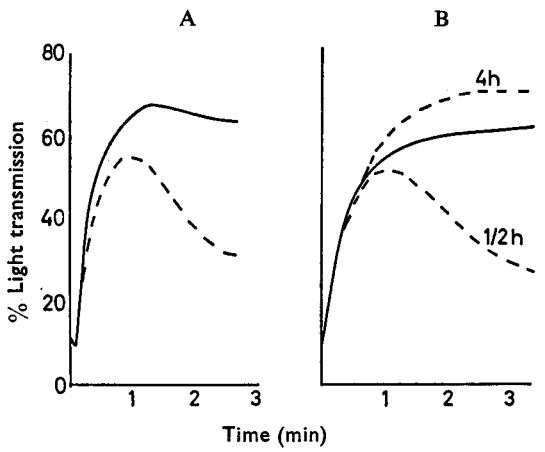

FIG. 1. A. *In vitro* effect of carbenicillin (160 μg ml^{-1}) on ADP-induced aggregation in platelet-rich plasma (—— control, – – – – with carbenicillin; ADP to 2 μM final concentration).

B. *In vitro* effect of carbenicillin (5 g i.v.) on ADP-induced aggregation in platelet-rich plasma (—— pre-carbenicillin, – – – – post-carbenicillin; ADP to 2 μM final concentration).

RESULTS

In vitro

Carbenicillin had no effect on the thrombin time, partial thromboplastin time, prothrombin time, platelet factor 3 availability and fibrinogen and plasminogen levels (Table 1).

Table 1. *Results of tests.*

	The effect of the addition of carbenicillin (20–1280 μg ml^{-1}) to pooled human plasma				The effect of the intravenous administration of 5 g carbenicillin to volunteers		
	pre-carbenicillin	Mean ± s.e. post-carbenicillin	pre-carbenicillin with 2 h incubation	Mean ± s.e. post-carbenicillin with 2 h incubation	Mean ± s.e. pre-injection	Mean ± s.e. 30 min after injection	Mean ± s.e. 4 h after injection
Prothrombin time (i) (s)	15·0	14·8 ± 0·1	12·1	12·1 ± 0·1	11·9 ± 0·2	11·9 ± 0·5	11·8 ± 0·2
Thrombin time (ii) (s)	24·9	24·7 ± 0·3	22·7	22·5 ± 0·4	17·0 ± 1·3	17·6 ± 1·6	18·7 ± 1·1
Partial thromboplastin (iii) time (s)	30·5	30·8 ± 0·3	30·5	30·5 ± 0·1	39·2 ± 1·6	40·5 ± 3·7	37·9 ± 2·15
Platelet factor 3 (iv) (s)	27·0	27·0 ± 0·4	23·4	23·5 ± 0·6	31·4 ± 1·4	32·2 ± 1·5	32·3 ± 3·3
Fibrinogen (v) (mg per 100 ml)	168	172 ± 4·6	206	168 ± 11·4	322 ± 34	306 ± 25	319 ± 31
Plasminogen (vi) (casein units)	2·6	2·6 ± 0·1	1·5	1·7 ± 0·1	2·5 ± 0·5	2·4 ± 0·5	2·5 ± 0·5
Euglobulin lysis (vii) time (min)	No change				384 ± 61	333 ± 78	250 ± 45
Fibrin plate lysis (viii) (mm)	No change				5 ± 1	6 ± 1	8 ± 1
Adhesiveness (%) (ix)	—	—	—	—	87 ± 1	90 ± 1	88 ± 2
Bleeding time (min) (x)	—	—	—	—	8 ± 1	—	8 ± 2

(i) One-stage prothrombin time. Method of Quick, Stanley-Brown & Bancroft (1935).
(ii) Thrombin time. Method of Ratnoff & Colopy (1954).
(iii) Partial thromboplastin time. Method of Langdell, Wagner & others (1953). Thrombofax (Ortho) as thromboplastin source.
(iv) Platelet factor 3 availability. Method of Spaet & Cintron (1965).
(v) Fibrinogen. Method of Ratnoff & Menzie (1951).
(vi) Plasminogen. Method of Remmert & Cohen (1949) as described by Alkjaersig, Fletcher & Sherry (1959).
(vii) Euglobulin lysis time. Method of Nilsson & Olow (1962).
(viii) Fibrin plate lysis. Method of Müllertz (1952).
(ix) Platelet adhesiveness. Method of Bowie, Owen & others (1969).
(x) Bleeding time. Method of Mielke, Kaneshiro & others (1969) using a standard incision with a template guide.

Euglobulin lysis time, fibrin plate lysis and factor XIII were also unaffected by addition of the drug. Carbenicillin had no immediate effect on platelet aggregation to ADP. Following pre-incubation for 2 h at 37° platelet aggregation to ADP was inhibited. This effect was apparent at all concentrations of the drug tested. A typical tracing showing the effect of carbenicillin on platelet aggregation to ADP is shown in Fig. 1A.

In vivo

The mean carbenicillin concentrations at 30 min and 4 h after injection were 354·5 ± 116 and 59·8 ± 18 μg ml^{-1} (mean ± s.d.) respectively.

Carbenicillin 30 min and 4 h after injection had no effect on prothrombin time, partial thromboplastin time, thrombin time, platelet factor 3 availability, fibrinogen and plasminogen levels, euglobulin lysis time, fibrin plate lysis and platelet adhesiveness. The bleeding time 4 h after injection was unaffected (Table 1).

No statistically significant effect on platelet aggregation in response to ADP, adrenaline or collagen was found in the six subjects. However, two of the six subjects 30 min after the injection showed marked disaggregation in response to a dose of ADP which previously had caused irreversible aggregation (Fig. 1B). In addition, two subjects showed disaggregation at 4 h.

One of the six subjects showed considerable shortening of euglobulin lysis time in samples taken at 30 min and 4 h after the injection, the time falling from 390 to 60 and 80 min respectively.

DISCUSSION

No constant effect of carbenicillin was demonstrated upon the haemostatic functions tested in normal subjects. The results showed that carbenicillin inhibited platelet aggregation in response to ADP *in vitro* after the drug had been incubated with plasma and that platelet function was similarly impaired in two subjects 30 min after and in two subjects 4 h after injection of the drug. However, the bleeding time was normal after 4 h in all six subjects and it seems unlikely that in the absence of any effect on the bleeding time the impairment of platelet function demonstrated would contribute to a bleeding tendency.

Carbenicillin was shown to have no effect on the other tests of haemostatic function performed *in vitro* or following administration to normal subjects. These results suggest that in the concentrations used, which are those likely to be obtained therapeutically, carbenicillin does not act as a direct inhibitor of haemostasis. The findings, however, do not exclude the possibility that carbenicillin might interfere with the synthesis of coagulation factors or alter fibrinolytic activity in human subjects exposed to the drug for periods longer than 6 h and the effects on platelet function might be significant in patients with uraemia where platelet function is already abnormal (Calahane, Johnson & others, 1958; Castaldi, Rozenberg & Stewart, 1966; Salzman & Neri, 1966; Horowitz, Cohen & others, 1967; Eknoyan, Wacksman & others, 1969).

Previous studies of the effects of carbenicillin on coagulation have shown conflicting results. Lurie and his colleagues (1970a,b) found prolongation of the prothrombin time and kaolin cephain clotting time in uraemic patients undergoing treatment with the drug, and during *in vitro* studies showed that high concentrations of carbenicillin

(6000 μg ml^{-1} and greater) produced a similar abnormality. However, such concentrations are many times greater than those recommended for therapeutic use, though they may be attainable in patients with renal failure treated with standard dosage regimes. McClure & others (1970) were unable to confirm these findings but showed that at concentrations of 700 μg ml^{-1} carbenicillin inhibited platelet aggregation *in vitro*. The results reported here are in agreement with the work of McClure & others (1970) in showing carbenicillin to affect platelet function, but they suggest that the effect produced is unlikely to provoke bleeding in patients treated with conventional doses of the drug.

Acknowledgements

We would like to thank Beecham Laboratories Ltd. for supplying carbenicillin and for carrying out assays of carbenicillin in plasma samples, and Mr. J. Leek and Mrs. A. Spencer for expert technical assistance. We are grateful to the Board of Governors of the United Leeds Hospitals for financial support.

REFERENCES

ACRED, P., BROWN, D. M., KNUDSEN, E. T., ROLLINSON, G. N. & SUTHERLAND, R. (1967). *Nature*, **215**, 25–30.

ALKJAERSIG, G. N., FLETCHER, A. P. & SHERRY, S. (1959). *J. clin. Invest.*, **38**, 1086–10095.

BODEY, G. P. & TERRELL, L. M. (1968). *J. Bact.*, **95**, 1587–1590.

BORN, G. V. R. (1962). *J. Physiol.*, **162**, 67–68P.

BOWIE, W. E. J., OWEN, C. A., THOMPSON, J. H. & DIDISHEIM, P. (1969). *Am. J. clin. Path.*, **52**, 69–77.

BRUMFITT, W., PERCIVAL, A. & LEIGH, D. A. (1967). *Lancet*, **1**, 1289–1293.

CALAHANE, S. F., JOHNSON, S. A., MONTO, R. W. & CALDWELL, J. M. (1958). *Am. J. clin. Path.*, **30**, 507–513.

CASTALDI, P. A., ROZENBERG, M. C. & STEWART, J. H. (1966). *Lancet*, **2**, 66–69.

EKNOYAN, G., WACKSMAN, S. H., GLUECK, H. I. & WILL, J. J. (1969). *New Eng. J. Med.*, **280**, 677–681.

HOROWITZ, H. E., COHEN, B. D., MARTINEZ, P. & PAPAYOANOU, M. F. (1967). *Blood*, 30, 331–340.

LANGDELL, R. D., WAGNER, R. H., BRINKHOUSE, K. H. & HILL, C. N. C. (1953). *J. Lab. clin. Med.*, **41**, 637–647.

LORAND, L. (1950). *Nature*, **166**, 694–695.

LURIE, A., OGILVIE, M., TOWNSEND, R., GOLD, C., MEYERS, A. M. & GOLDBERG, B. (1970a). *Lancet*, **1**, 1114–1115.

LURIE, A., GOLD, C. H., MEYERS, A. M. & GOLDBERG, B. (1970b). *Ibid.*, **2**, 422–423.

MARKS, M. I. & EICKHOFF, T. C. (1970). *Ann. intern. Med.*, **73**, 179–187.

MCCLURE, P. D., CASSERLY, J. G., MONSIER, C. & CROZIER, D. (1970). *Lancet*, **2**, 1307–1308.

MIELKE, C. H., KANESHIRO, M. M., MAHER, I. A., WEINER, J. H. & RAPAPORT, S. I. (1969). *Blood*, **34**, 204–215.

MÜLLERTZ, S. (1952). *Acta physiol. scand.*, **25**, 93–100.

NILSSON, I. M. & OLOW, B. (1962). *Acta chir. scand.*, **123**, 247–266.

QUICK, A., STANLEY-BROWN, M. & BANCROFT, F. W. (1935). *Am. J. med. Sci.*, **190**, 501–511.

RATNOFF, O. & COLOPY, J. E. (1954). *J. clin. Invest.*, **33**, 1175–1182.

RATNOFF, O. D. & MENZIE, C. A. B. (1951). *J. Lab. clin. Med.*, **37**, 316–320.

REMMERT, L. F. & COHEN, P. P. (1949). *J. biol. Chem.*, **181**, 431–448.

SALZMAN, E. W. & NERI, L. L. (1966). *Thromb et diath. haemorr.*, **15**, 84–92.

SPAET, T. H. & CINTRON, J. (1965). *Brit. J. Haemat.*, **11**, 269–275.

WAISBREN, B. A., EVANI, S. V. & ZIEBERT, A. P. (1971). *J. Am. med. Ass.*, **217**, 1243.

YUDIS, M., MAHOOD, W. H. & MAXWELL, R. (1972). *Lancet*, **2**, 599.